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Growth of catalase A and catalase T deficient mutant strains of *Saccharomyces cerevisiae* on ethanol and oleic acid

Growth profiles and catalase activities in relation to microbody proliferation

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Abstract. The parental strain (A^+T^+) of *Saccharomyces cerevisiae* and mutants, deficient in catalase T (A^+T^-), catalase A (A^-T^+) or both catalases (A^-T^-), grew on ethanol and oleic acid with comparable doubling times. Specific activities of catalase were low in glucose- and ethanol-grown cells. In the two oleic acid-grown A^+ -strains (A^+T^+ and A^+T^-) high catalase activities were found; catalase activity invariably remained low in the A^-T^+ strain and was never detected in the A^-T^- strain. The levels of β -oxidation enzymes in oleic acid-grown cells of the parental and all mutant strains were not significantly different. However, cytochrome C peroxidase activity had increased 8-fold in oleic acid grown A^- strains (A^-T^+ and A^-T^-) compared to parental strain cells. The degree of peroxisomal proliferation was comparable among the different strains. Catalase A was shown to be located in peroxisomes. Catalase T is most probably cytosolic in nature and/or present in the periplasmic space.

Key words: *Saccharomyces cerevisiae* — Catalase A — Catalase T — β -Oxidation — Microbodies — H_2O_2 -Metabolism

Catalase is an important enzyme in the decomposition of intracellular hydrogen peroxide (H_2O_2). In yeasts enhanced levels of this enzyme are found during growth of cells on various carbon and/or nitrogen sources, the metabolism of which is mediated by peroxisome-borne H_2O_2 -producing oxidases. Both these oxidases and catalase are localized in the same subcellular compartments, called peroxisomes (Zwart 1983; Veenhuis and Harder 1987a, b). As a result, synthesis of these enzymes is generally paralleled by the development of peroxisomes. A striking example of this peroxisomal proliferation is encountered in methanol-grown cells of the yeast *Hansenula polymorpha* (Veenhuis et al. 1979).

In *Saccharomyces cerevisiae* peroxisomal proliferation is induced by oleic acid, paralleled by the induction of catalase A (Skoneczny et al. 1988) and the different enzymes of the β -oxidation cycle, which are localized in these organelles (Veenhuis et al. 1987).

Recent studies (Guiseppin et al. 1988) indicated that catalase probably is not indispensable for the proper functioning of peroxisome-mediated oxidative metabolism. This is suggested by the finding that catalase-deficient mutant strains of *H. polymorpha* are able to consume methanol during growth in continuous cultures on glucose/methanol mixtures, although the organism could not grow on methanol alone. The H_2O_2 produced in this mutant is probably decomposed by a mitochondrial enzyme, cytochrome C peroxidase (CCP), which was present in enhanced levels under these conditions (Verduyn et al. 1988). Furthermore, in wild type *Saccharomyces cerevisiae* enhanced levels of CCP but not of catalase were found in continuous cultures on mixtures of glucose and H_2O_2 (Verduyn et al. 1988). These results indicate that in yeasts, apart from catalase, other mechanisms involved in H_2O_2 -metabolism may be operative.

For this reason we studied the adaptation of different catalase-deficient mutants of *S. cerevisiae* to various growth conditions. In this yeast two catalases are present, namely the peroxisomal catalase A (Atypical) (Skoneczny et al. 1988) and a non-peroxisomal catalase T (Typical). The two catalases are encoded by different genes, CTA1 and CTT1 respectively (Taczuk et al. 1985). Of the different strains studied (listed in Table 1) we compared both growth characteristics and catalase levels, induced by ethanol or oleic acid, in relation to peroxisomal proliferation. The results of these experiments are presented in this paper.

Materials and methods

Organisms and growth

The parental and different catalase-mutant strains of *Saccharomyces cerevisiae* used in this study are listed in Table 1. Cells were grown

Table 1. Different strains of *Saccharomyces cerevisiae* used in this study

Strain	Genotype	Name
SP4	α ,leu1,arg4	A ⁺ T ⁺
DCT1-2C	α ,leu1,arg4,ctt1-1	A ⁺ T ⁻
DCT3-8D	α ,leu1,cta1-2	A ⁻ T ⁺
DCT11-3A	α ,leu1,leu2-3,leu2-112, his4-519,cta1-2,ctt1-4	A ⁻ T ⁻

For details see Taczky et al. (1985)

in batch cultures at 30°C in mineral media as described previously (Veenhuis et al. 1987). As carbon sources (a) 0.1% (v/v) oleic acid in the presence of 0.1% (w/v) yeast extract and 0.02% (v/v) Tween 80 or (b) 0.5% (v/v) ethanol were used.

Growth was followed by measuring the absorbance (A) at 660 nm. Oleic acid was dissolved by adding Teepol [2% (w/v) final concentration] prior to measurements.

Analytical procedures

Cell free extracts were prepared as described previously (Veenhuis et al. 1986). Catalase was assayed as described by Lück (1963), the β -oxidation enzymes acyl CoA oxidase, bifunctional enzyme (comprising enoyl CoA hydratase and L-3-hydroxyacyl CoA dehydrogenase) and 3-oxoacyl CoA thiolase as described by Kionka and Kunau (1985) and cytochrome C peroxidase as described by Verduyn et al. (1988). Protein concentrations were determined as described by Lowry et al. (1951) using bovine serum albumin as standard. Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was performed as described by Laemmli (1970). Western blotting was carried out by the Protoblot immunostaining method (Promega Biotec); specific antibodies against catalase T were kindly provided by Prof. Dr. H. Ruis, University of Vienna, Austria.

(Immuno)cytochemistry

Catalase activity was localized with the conventional DAB-based method described previously (Van Dijken et al. 1975). Immunolabeling experiments were performed on ultrathin sections of Lowicryl-embedded cells (Douma et al. 1985) with antibodies against catalase T, mentioned above and protein A/gold.

Fixation and postfixation methods

Whole cells were fixed in 1.5% (w/v) potassium permanganate (KMnO₄) for 20 min at room temperature and prepared for electron microscopy as described before (Veenhuis et al. 1979). The average number and relative volume of microbodies was determined as described previously (Veenhuis et al. 1979).

Results

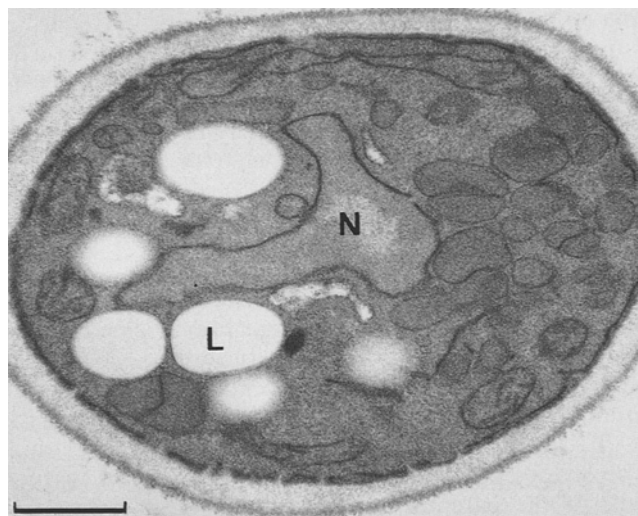
Growth profiles

The growth patterns obtained with the *S. cerevisiae* parental strain (A⁺T⁺) and the different catalase-deficient strains on ethanol revealed that the A⁻-strains showed prolonged lag phases compared to A⁺-strains after a shift

Table 2. Catalase activities in crude extracts prepared from the parental and catalase-deficient mutant cells grown on different carbon sources

Strain	Glucose	Ethanol	Oleic acid
A ⁺ T ⁺	2.6	3.8	34.8
A ⁺ T ⁻	1.9	3.4	51.3
A ⁻ T ⁺	0.9	0.7	1.5
A ⁻ T ⁻	0.0	0.0	0.0

Catalase activity is expressed as $\Delta E_{240}/\text{min} \cdot \text{mg protein}$. Cells were harvested from the late exponential (ethanol and oleic acid) or stationary growth phase (glucose)

**Fig. 1.** Survey of the parental strain (A⁻T⁺) of *S. cerevisiae* to show the peroxisomal morphology and proliferation in cells from the stationary growth phase. L, lipid droplet; N, nucleus. Bar: 0.5 μm .

of cells from glucose to ethanol; however, after growth had started all four strains studied grew with equal doubling times ($t_d = 4$ h). The behaviour of these strains after transfer of cells into oleic acid-containing media was largely comparable: after a relative short lag all strains grew with identical doubling times ($t_d = 6.5$ h).

Biochemistry

Catalase activities were determined in crude extracts prepared from the parental strain and the different catalase-deficient mutant strains, grown on different carbon sources. The results, summarized in Table 2 show that in A⁻T⁻-cells catalase activity was invariably absent. In extracts from glucose-grown cells of the three other strains catalase activity was low. Lowest activities were measured in the A⁻T⁺-strain whereas slightly enhanced levels were detected in extracts from ethanol-grown cells of both A⁺-strains. However, high catalase activities were found in oleic acid-grown cells of both A⁺-strains; in contrast, in the A⁻T⁺-strain catalase activities remained low. These results indicate that the synthesis of catalase A is strongly induced by oleic acid, while catalase T apparently is constitutively present.

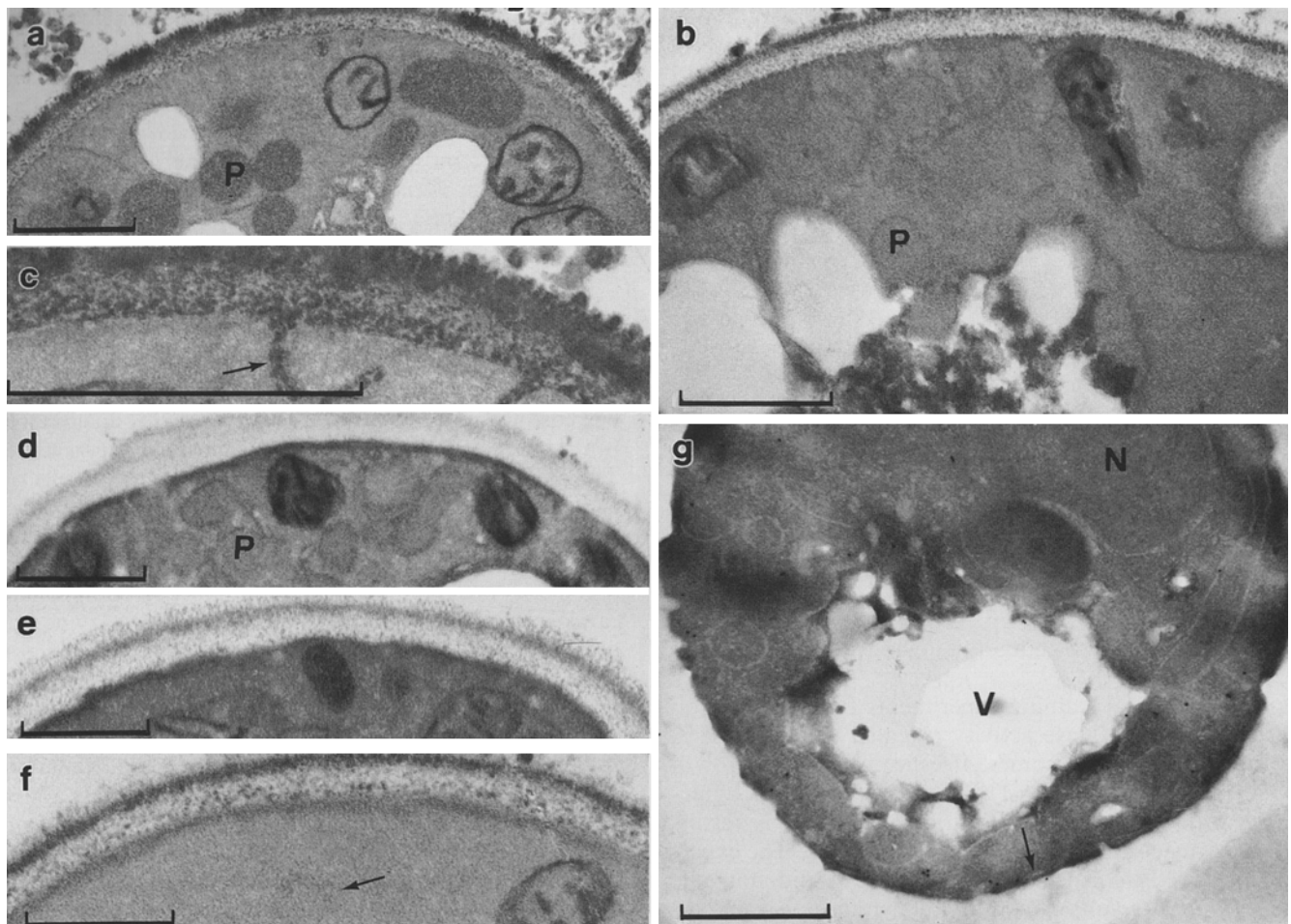


Fig. 2a–g. Cytochemical demonstration of the subcellular sites of catalase activity. In the parental strain (A^+T^+) all peroxisomes (P) are positively stained (**a** oleic acid). Specific staining was also detected in the inner layer of the cell wall and the periplasmic space; this staining was more intense after prolonged incubations (**c** *arrow*; ethanol); in control experiments in the presence of aminotriazole the peroxisomal staining was fully blocked whereas still minor reaction products were seen in the cell wall (**b** oleic acid). Staining of both

cell wall and peroxisomes was absent in the A^-T^- mutant strain (**d** oleic acid). In the A^+T^- strain solely peroxisomes stained (**e** ethanol), whereas in A^-T^+ strains peroxisomes were unstained but cell wall staining was still evident (**f** ethanol). **g** Immuno electron micrograph of an oleic acid-grown cell of the parental strain (A^+T^+) incubated with anti-catalase T and protein A/gold; the low labeling is scattered over the cytosol and infrequently also located in the periplasmic space (*arrow*). Bars: 0.5 μ m. V , vacuole; N , nucleus

In oleic acid-grown cells of both A^- -strains the levels of cytochrome C peroxidase activity had increased approximately 8-fold, compared to the parental (A^+T^+) strain (3.5 U/mg protein in A^+T^+ , compared to 28.5 U/mg protein in the A^-T^- strain). The activities of the β -oxidation enzymes measured (acyl CoA oxidase, bifunctional enzyme and the thiolase activity) showed no significant differences among the various strains and were present in amounts as described before for wild type cells (for detailed data see Veenhuis et al. 1987).

Electron microscopy

The proliferation of peroxisomes in both the parental and the mutant strains was studied on ultrathin sections of $KMnO_4$ -fixed cells. Serial sectioning experiments revealed that ethanol-grown cells contained only a single or few (generally 1–3) small microbodies, slightly in-

creased in size (diameters up to 0.2 μ m) compared to the organelles present during growth of cells on glucose (Veenhuis et al. 1987). However, in all strains peroxisomal proliferation was strongly induced during incubation of cells in oleic acid-containing media. In cells from the mid-exponential growth phase ($A_{660} = 0.8–0.9$) of all the strains studied many peroxisomal profiles were observed, which were frequently partly clustered and scattered throughout the cytoplasm. Compared to the glucose-inoculum cells the size of the organelles had considerably increased; in all strains the morphology of the organelles was essentially similar with diameters up to 0.7 μ m. Also the average number of microbodies (varying from 2.3–2.5 peroxisomal profiles per cell in ultrathin sections) and their relative volume fractions (ranging from 9.9–10.4% of the cytoplasmic volume) did not vary significantly among all strains. In exponentially growing cells the peroxisomes were generally larger in size when compared to cells from the stationary growth phase (Fig. 1; for

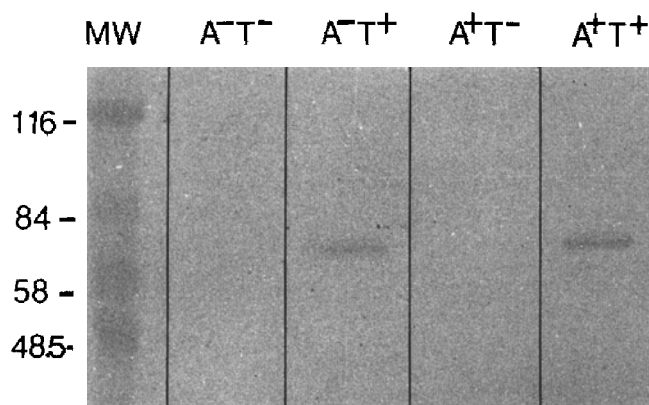


Fig. 3. Western blot, prepared from cell free extracts of ethanol-grown parental and catalase-deficient strains using antibodies against catalase T. Equal amounts of protein were loaded on each lane. Specific bands at approximately 60000 were only detected in blots of the two strains containing catalase T (A^+T^+ and A^-T^+) and absent in the catalase T-deficient strains (A^-T^- and A^+T^-)

detailed data on exponentially growing cells see Veenhuis et al. 1987).

Cytochemical staining experiments for the detection of the sites of catalase activity revealed that catalase A is localized in peroxisomes, irrespective of the growth conditions (Fig. 2a, e). This localization is in agreement with earlier — (immuno)cytochemical — results (Skoneczny et al. 1988; Veenhuis et al. 1987). The experiments for the localization of catalase T did not give unequivocal results. In immunocytochemical experiments, using specific antibodies against catalase T and protein A/gold, low labeling intensities were found mainly in the cytosol (Fig. 2g), indicating the localization of the enzyme in this compartment. The presence of the enzyme was confirmed in Western blots (Fig. 3). Cytochemically, using the conventional DAB-method, cytosolic staining was not significant and could not clearly be discriminated from the background density of the cytosol in control experiments, lacking the H_2O_2 substrate (not shown) or performed in the presence of aminotriazole as an inhibitor of catalase activity (Fig. 2b). However, in the majority of A^+T^+ -parental cells (approximately 70%–80%) staining deposits were observed in the periplasmic space and/or inner layer of the cell wall (Fig. 2a). The intensity of this staining increased upon prolonged incubation times (Fig. 2c). Similar staining patterns were found in the A^-T^+ -strain (Fig. 2f), but were rare in the A^+T^- (Fig. 2e) and absent in the A^-T^- -strain (Fig. 2d). The intensity of this staining was strongly sensitive to aminotriazole but not fully prevented — as was the case for catalase A — after incubations in the presence of this compound (Fig. 2b). We interpret these results such that catalase T is mainly cytosolic in nature but may also be present in the periplasmic space. The absence of significant reaction products in the cytosol most probably must be explained by the low levels of the enzyme together with dilution of the reaction products in this compartment, which renders these activities below the limit of detection of the

cytochemical method. This was furthermore indicated by the finding that in catalase T-overproducing transformants of *H. polymorpha* (Hansen and Roggenkamp 1989) the cytosolic staining could readily be detected (I. Keizer-Gunnink and M. Veenhuis, unpublished results). This dilution effect however is limited in the periplasm thus explaining the visualization of the reaction products in this compartment.

Discussion

In the present work we showed that the presence of peroxisomal catalase is not a prerequisite for an effective functioning of peroxisomes in *Saccharomyces cerevisiae*. This is indicated by the finding that growth of cells on oleic acid — and therefore also the oxidative metabolism of this substrate — can occur independent of the presence of peroxisomal catalase A. Interestingly, catalase T apparently is not the first enzyme of choice to complement catalase A-deficiency since the activity of this enzyme was not influenced by growth conditions and even did not increase in the A^-T^+ -strain during growth on oleic acid. Our results indicate that in these cells the mitochondrial enzyme cytochrome C peroxidase may play a significant role in intracellular H_2O_2 -metabolism. These results are in line with earlier observations that extracellularly added H_2O_2 does not influence catalase levels in *S. cerevisiae* (Veenhuis et al. 1987; Verduyn et al. 1988) but instead causes an increase in cytochrome C peroxidase activity (Verduyn et al. 1988). In *Hansenula polymorpha*, which contains one — peroxisomal — catalase, activity of this enzyme is essential to support growth of the organism on methanol (Verduyn et al. 1988). These studies furthermore indicated that in wild type cells catalase could only effectively compete with mitochondrial cytochrome C peroxidase if H_2O_2 is generated at the sites where catalase is located, namely in the peroxisomal matrix. These results are in agreement with recent findings of Hansen and Roggenkamp (1989) who showed that heterologous expression of catalase A from *S. cerevisiae* in catalase-deficient mutants of *H. polymorpha* resulted in import and assembly of the protein into peroxisomes and functional complementation in that the transformed cells could grow again on methanol. However, functional complementation was not achieved after expression of the *S. cerevisiae* catalase T gene, the product of which accumulated in the cytosol (Hansen and Roggenkamp 1989).

The reasons why one peroxisome-bound metabolic pathway is fully operative in the absence of catalase (oleic acid by *S. cerevisiae*) whereas the other is not (methanol by *H. polymorpha*) is not yet clear. In *H. polymorpha* this may be related to a possible inactivation of alcohol oxidase by H_2O_2 generated during methanol oxidation (Kato et al. 1976). An additional explanation may include the relatively high energetic disadvantage of H_2O_2 decomposition by cytochrome C peroxidase (Verduyn et al. 1988) during methanol utilization compared to utilization of oleic acid.

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